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TITLE: Improved Healing of Large, Osseous, Segmental Defects by Reverse Dynamization: Evaluation in a Sheep Model

PRINCIPAL INVESTIGATOR: Christopher H. Evans

CONTRACTING ORGANIZATION: Mayo Clinic
Rochester, MN 55905

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Although this is officially an annual report, the release of the funds was delayed until July 2014 because of the PI's transfer from Beth Israel Deaconess Medical Center to the Mayo Clinic. Activities since then have succeeded in obtaining ICACUC and ACURO approvals, which was the first task in the statement of work, months 0-3. We have also initiated work on the second task, months 3-6 in which the design of the adjustable stiffness fixators will be finalized and the manufacturing initiated. One such fixator has been tested on a cadaveric sheep tibia. In the unlocked, loose position, the axial stiffness of the tibia and fixator was 102 N/mm. In the locked, stiff position it was 218 N/mm. This result is better than expected and close to the target of a 2.5-fold increment suggested by our previous studies using rats. This aspect of the project is the present focus of attention, and additional cadaver legs will be tested in due course.

15. SUBJECT TERMS
Sheep; tibia; external fixator; axial stiffness

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1. Introduction

Large segmental defects in the long bones do not heal well and are a major clinical problem [1]. Battlefield warriors are at particular risk of such injuries.

Recombinant, human bone morphogenetic protein-2 (rhBMP-2), the active ingredient of the product INFUSE®, is used by surgeons to assist the healing of large osseous lesions but the clinical results have been disappointing [2]. Moreover, rhBMP-2 is very expensive. Therefore improving the efficacy of rhBMP-2 promises to provide better bone healing at a lower cost. The present grant funds a project designed to determine whether this can be achieved by modulating the mechanical environment under which healing takes place.

It is well established that bone healing is influenced by the mechanical exnvironment. Segmental defects may be stabilized mechanically by an external fixator. There has been much interest in the concept of dynamization, whereby the defect is first stabilized rigidly to initiate healing and then subjected to axial motion (dynamization) to promote the subsequent stages of healing and maturation [3].

In research funded by a CDMRP Idea Development Award, we used a rat segmental defect model to show that healing in response to rhBMP-2 could be accelerated and improved by “reverse dynamization” in which the fixator is first applied in a loose configuration (114 N/mm) and then stiffened (254 N/mm) once bone formation had started [4, 5].

The present research will determine whether reverse dynamization is also effective in sheep, as a stepping stone towards human, clinical trials.

2. Keywords

Bone healing; segmental defect; reverse dynamization; sheep; external fixator

3. Overall Project Summary

Although this is officially an annual report, because of the PI’s transfer from Beth Israel Deaconess Medical Center to the Mayo Clinic, the funding did not begin until July 2013. While the transfer was being implemented, the first task of the Statement of Work (Obtain regulatory approval for sheep studies from IACUC and ACURO) was accomplished (see appendix).

Since then, work has started on the second and third tasks (Finalize design and manufacture of adjustable stiffness fixators ; Optimize and characterize external fixators**).**

A 3 mm tibial defect was created in the leg of a cadaveric sheep, and stabilized with an experimental external fixator in the “loose” configuration. The axial stiffness of this construct was then measured as 102 N/mm. The external fixator was then altered to the “stiff” configuration and its axial stiffness again measured. This determined an axial stiffness of 218 N/mm.

These values are encouragingly similar to the values of 114 N/mm and 254 N/mm that proved successful in the rat.

4. **Key Research Accomplishments**

a) Obtaining IACUC and ACURO approval.
b) Designing and constructing the first prototype external fixator
c) Successfully determining the axial stiffness of the fixator in loose and stiff configuration, determining values close to the target values.

5. **Conclusion**

Because these customized external fixators are central to the success of this project, these accomplishments provide optimism that the proposed research can be achieved as planned.

Our next task is to refine and finalize iteratively the fixator design, thereby positioning ourselves to begin in vivo experiments in sheep in year 2.

6. **Publications, Abstracts and Presentations**

None

7. **Inventions, Patents and Licenses**

None

8. **Reportable Outcomes**

A prototype adjustable external fixator

9. **Other Achievements**

None

10. **References**

1 Pollak, A.N. and Ficke, J.R. (2008) Introduction. Extremity war injuries: challenges in definitive reconstruction. *The Journal of the American Academy of Orthopaedic Surgeons* 16, 626-627
2 Carragee, E.J.*, et al.* (2011) A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned. *The spine journal : official journal of the North American Spine Society* 11, 471-491
3 De Bastiani, G.*, et al.* (1984) The treatment of fractures with a dynamic axial fixator. *The Journal of bone and joint surgery. British volume* 66, 538-545
4 Glatt, V.*, et al.* (2012) Improved healing of large segmental defects in the rat femur by reverse dynamization in the presence of bone morphogenetic protein-2. *The Journal of bone and joint surgery. American volume* 94, 2063-2073
5 Glatt, V.*, et al.* (2012) Design, characterisation and in vivo testing of a new, adjustable stiffness, external fixator for the rat femur. *European cells & materials* 23, 289-298; discussion 299

11. **Appendices**

a) IACUC approval letter
b) ACURO approval letter
c) Quad Report

MAYO CLINIC

Institutional Animal Care and Use Committee

200 First Street SW
Rochester, MN 55905
507-284-2511

March 13, 2014

To Whom It May Concern:

RE: Institutional Animal Care and Use Committee Approval

The following application was reviewed and approved by this Institution's Animal Care and Use Committee on March 5, 2014:

Protocol Title: "Improved healing of large, osseous, segmental defects by reverse dynamization: evaluation in a sheep model"

Dr. C. Evans, Principal Investigator, Mayo Clinic

This Institution has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare. The assurance number is A3291-01.

Sincerely,

Jason H. Pitzen
Administrator,
Institutional Animal Care and Use Committee

JHP:edh
cc: Dr. C. Evans
Mr. R. Wetmore
(A3414)

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MD 21702-5012

REPLY TO
ATTENTION OF

June 18, 2014

Director, Office of Research Protections
Animal Care and Use Review Office

Subject: Review of USAMRMC Proposal Number OR120192, Award Number W81XWH-13-1-0324 entitled, "Improved Healing of Large, Osseous, Segmental Defects by Reverse Dynamization: Evaluation in a Sheep Model"

Principal Investigator Christopher Evans
Mayo Clinic, Arizona
Scottsdale, AZ

Dear Dr. Evans:

Reference: (a) DOD Instruction 3216.01, "Use of Animals in DOD Programs"
(b) US Army Regulation 40-33, "The Care and Use of Laboratory Animals in DOD Programs"
(c) Animal Welfare Regulations (CFR Title 9, Chapter 1, Subchapter A, Parts 1-3)

In accordance with the above references, protocol OR120192 entitled, "Improved Healing of Large, Osseous, Segmental Defects by Reverse Dynamization: Evaluation in a Sheep Model," IACUC protocol number A3414 is approved by the USAMRMC Animal Care and Use Review Office (ACURO) for the use of sheep and will remain so until its modification, expiration or cancellation. This protocol was approved by the Mayo Clinic and Foundation, Rochester IACUC.

When updates or changes occur, documentation of the following actions or events must be forwarded immediately to ACURO:

- IACUC-approved modifications, suspensions, and triennial reviews of the protocol (All amendments or modifications to previously authorized animal studies must be reviewed and approved by the ACURO prior to initiation.)
- Reports to OLAW involving this protocol regarding

  a. any serious or continuing noncompliance with the PHS Policy;
  b. any serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals; or
  c. any suspension of this activity by the IACUC

- USDA or OLAW regulatory noncompliance evaluations of the animal facility or program
- AAALAC, International status change (gain or loss of accreditation only)

Throughout the life of the award, the awardee is required to submit animal usage data for inclusion in the DOD Annual Report on Animal Use. Please ensure that the following animal usage information is maintained for submission:

- Species used (must be approved by this office)
- Number of each species used
- USDA Pain Category for all animals used

For further assistance, please contact the Director, Animal Care and Use Review Office at (301) 619-2283, FAX (301) 619-4165, or via e-mail: usarmy.detrick.medcom-usamrmc.other.acuro@mail.mil.

NOTE: Do not construe this correspondence as approval for any contract funding. Only the Contracting Officer or Grant Officer can authorize expenditure of funds. It is recommended that you contact the appropriate Contract Specialist or Contracting Officer regarding the expenditure of funds for your project.

Sincerely,

For

GOODWIN.SUSAN.DOORE.1047618866

Bryan K. Ketzenberger, DVM, DACLAM
Colonel, US Army
Director, Animal Care and Use
Review Office

Copies Furnished:
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Dr. Christopher Evans, Mayo Clinic and Foundation, Rochester
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Dr. Randall E. Wetmore, Mayo Clinic and Foundation, Rochester
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